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Efficacy of two inducing agents PG and DoM+SGnRH in the induced breeding of the major carp, kalibaus (*Labeo calbasu*)

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Abstract: An experiment on the induced breeding of the endangered fish, *Labeo calbasu* (Hamilton-Buchanam) was conducted in the Fish Seed Multiplication Farm,Rajshahi to know the efficacy of two inducing agents (PG and DoM+SGnRH). Three breeding trials of each inducing agent were performed. A total of 24 females weighing from 1.5 kg to 2 kg were given an initial and a resolving dose of 1.5 mg and 6 mg PG extract per kg body weight respectively as treatment-1. On the other hand, a total of 24 females weighing from 1.5 kg to 2 kg were given a single dose of 12 mg DoM + SGnRH/kg as treatment-2. In case of treatment-1, 12 males weighing from 1.5 kg to 1.95 kg were administered a single dose of 1.5 mg PG/kg body weight during resolving dose of female. In treatment-2, 12 males weighing from 1.5 kg to 1.8 kg were administered 3 mg DoM+SGnRH /kg body weight during initial dose of females. In treatment-1, the time interval between initial and resolving dose was 5 hours and ovulation occurred in all the injected females within 6 hours after resolving dose. Ovulation occurred within 6 to 8 hours after the injection of inducing agents for treatment-2. The mean rates of ovulation, fertilization and hatching were 100%, 77.36% and 74.5% respectively in treatment-1. On the contrary, the mean rates of ovulation, fertilization and hatching were 83.33%, 63.83% and 59.66% in treatment-2. Hatchery produced fry were reared in nursery pond for 40 days. In nursery pond. Flour, oil cake and wheat bran were applied as nursery feeds. Both the inducing agents were effective in respect of overall breeding performance. But the best results were obtained with PG although in case of DoM+SGnRH complete breeding takes place within short time with less labour and cost than that of PG.

Key words: *Labeo calbasu*,pituitary extract,ovaprim, induced breeding.

Introduction

In Bangladesh culture of carps is a very old practice. In China, carp culture is being practised since long time ago. The carp culture improves the social and economic status of farmers by adopting new scientific technology for breeding (Nandeesha and Rao, 1989).

The major problem in carp culture is the non-availability of quality fish seed.In early years fish seeds were collected from rivers by cloth happas. But this technique was unsafe as with the collection of carp seeds, some seeds of predatory fishes would have also been collected .Chaudhary and Alihunhi (1957) for the first time successfully carried out the spawning of major carps with induced breeding by pituitary extracts. According to Ahmed (1945), Alikunhi *et al*. (1960), Chaudhnri, 1960), Ali (1967), Sinha (1971) and Haque (1975), Alam *et. al*. (1999), Bhuiyan *et. al*. (2008), the PG influences the spawning of carp fishes. Such findings were observed by Lin (1965) and Tang *et al*. 1963) in Chinese carps.

In Bangladesh, the successful induced spawning was first done by Ali (1967) in carps through hypophysation. Other workers who worked on the induced breeding of carps include Haque, 1975; Islam & Chawdhury, 1976; Alam, 1983 and Ahmed, 1983. DoM+SGnRH was used, as a substitute for pituitary gland but it could not get the success as it was thought. The present investigation was conducted to know the comparative efficacy of the pituitary gland (PG) and Domperidome (Dom) + gonadoreleasing hprmone (SGnRH) on the carp, *Labeo calbasu*

Material and Methods

The present study was conducted with a view to knowing the comparative efficacy of the breeding performance of *Labeo calbasu* with two inducing agents PG hormone and DoM+SGnRH. Nursery management of this species was also conducted.

Brood stock rearing was conducted in the ponds of Fish Seed Multiplication Farm,Rajshahi.

For induced breeding of *Labeo calbasu* brood fishes were collected from the brood fishes rearing pond of the hatchery complex at 1:1 ratio. In this experiment three breeding trials with PG hormone and three breeding trials with DoM+SGnRH were performed, respectively as Treatment 1 and Treatment 2 to find out the comparative outcomes of these two inducing agents. Each trial was conducted by using 8 pairs of brood fishes. The dehydrated carp pituitary glands (PG) and DoM+SGnRH were collected from the local market in preserved condition in air tight vials used as an inducing agents. An electronic balance (College HP-TC 11, China) was used to weigh the required amount of PG and DoM+SGnRH by using the following formula-

$$\text{Weight of inducing agent (mg)} = \frac{W_t - P_t}{1000}$$

Where,

W_t represents total body weight (g) of all the fishes to be injected and

P_t represents the rate in mg inducing agents (PG and DoM+SGnRH) to be injected per kg body weight under a particular treatment.

The weighed PG was transferred to a tissue homogenizer and thoroughly crushed. The crushed PG was then diluted by distilled water and was centrifuged by a centrifuge machine for precipitation. The following ratio of inducing agents and water were maintained in order to prepare extracts. In treatment 1 the ratio of PG and water was 1 mg: 2.5 ml.In case of treatment 2 the ratio of DoM+SGnRH and water was 1mg:2ml

In case of treatment-1, the females were injected with 1.5mg PG/kg body weight as initial dose and 6 mg PG/kg body weight as resolving dose. On the other hand, males were administered at the rate of 1.5 mg PG/kg body weight. On the contrary in case of treatment-2, the females and males were injected DoM+SGnRH at the rate of 12 mg/kg body weight and 3 mg/kg body weight respectively. For determination of fertilization and hatching rates approximately 100 eggs were placed in plastic bowls of

1.25 liter capacity with three replications each having water flow from porous PVC pipe and outlet facility. At first the number of fertilized and unfertilized eggs of each bowl was counted with naked eyes. After approximately 18-30 h of fertilization, it was observed that hatching was almost complete and the number of hatchlings in each bowl was counted.

The following breeding parameters were recorded:

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of female fish injected}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized+unfertilized)}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

Results

After preparation of inducing agent extracts, it was injected to brood fishes. The fishes were caught carefully by scoop net and kept in sponge. Inducing agents was then injected near the pectoral fin base. The amount of PG and DoM + SGnRH solution for each fish was determined before according to the body weight of the brood fishes.

Doses of inducing agents for male and female broods

In this experiment, similar doses of PG extract were applied in different breeding trials as treatment-1 and similar doses of DoM+SGnRH were applied in different breeding trials as treatment-2. The doses of both solution (PG and DoM+SGnRH) are demonstrated in the following tables:

Table-1. Treatment-1 with PG for male and female brood fishes of *Labeo calbasu* in three breeding trials.

Trials	Pairs of brood fishes	Weight of brood fishes (kg)	Initial dose of PG (mg/kg body weight of fishes)	Time interval (hours)	Resolving dose of PG (mg/kg body weight of fishes)	Ovulation (hours)
Trial-1	8	Female=1.7±0.2 Male=1.65±0.1	Female=1.5	5.30	Female=6 Male=1.5	6.30
Trial-2	8	Female=1.8±0.1 Male=1.6±0.1	Female=1.5	5.15	Female=6 Male=1.5	6.15
Trial-3	8	Female=1.8±0.2 Male=1.75±0.2	Female=1.5	5	Female=6 Male=1.5	6

Table-2: Treatment-2 with DoM+SGnRH for male and female brood fishes of *Labeo calbasu* in three breeding trials

Trials	Pairs of brood fishes	Weight of brood fishes (kg)	Dose of DoM+SGnRH (mg/kg body weight of fishes)	Ovulation (hours)
Trial-1	8	Female=1.7±0.2 Male=1.6±0.1	Female= 12 Male=3	8
Trial-2	8	Female=1.8±0.1 Male=1.65±0.2	Female= 12 Male=3	6
Trial-3	8	Female=1.8±0.15 Male=1.6±0.1	Female= 12 Male=3	7

After administration of the inducing agents, the brood fishes were again released in the rectangular tank.

Collection of fertilized eggs and transferring to hatching tank

The fishes were removed from the rectangular tank when the ovulation was completed. Stripping was not required because the fertilization occurred in the circular tank. The fertilized eggs were collected from the outlet of circular tank with a net where the eggs came with water flow. The fertilized eggs were transferred into mini circular hatching tank with sufficient care. The mini circular tank was previously filled with filtered pond water to minimize the temperature difference and environmental shock. Continuous flow of water was maintained for aeration.

Ovulation rate

The average ovulation rate of the three breeding trials in case of treatment-1 and treatment-2 are shown respectively in Table-3 and Table-4. In Treatment-1, mean ovulation rate was (100%) in all the three trials. On the contrary, in treatment-2, the mean ovulation rate was (83.34%) recorded in the three trials. The highest ovulation rate (100%) was found in all the trials of treatment-1. The lowest ovulation rate (75%) was found in trial-I for Treatment-2. The graphical presentation of ovulation rate for both treatments are shown in Fig.1.

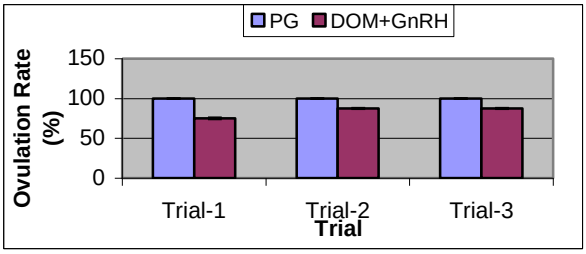

Fig-1. Ovulation rate (%) of *Labeo calbasu* in different breeding trials

Fertilization rate

In case of treatment-1 and Treatment-2 the average fertilization rate of three trials are shown respectively in Table 3 and Table 4. In treatment-1 the mean fertilization rate was 77.34% recorded in the three trials. On the other hand, in case of treatment-2 the mean rate of fertilization was 63.83 recorded in the three trials. The highest fertilization rate was 80% recorded in trials 3 followed by trial-1 and trial-2 (75% and 77%, respectively) for treatment-1. The lowest fertilization rate was 62.5% recorded in trial-2 for treatment-2. Graphical presentation of fertilization rate for both treatments are shown in Fig-2.

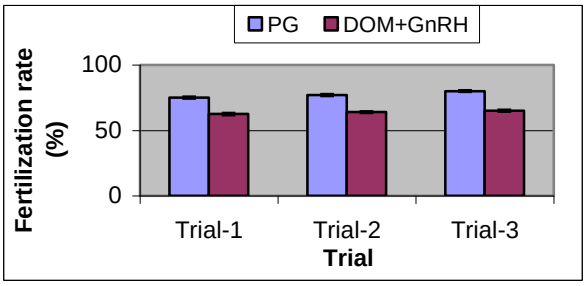

Fig.2. Fertilization rate (%) of *Labeo calbasu* in different breeding trials

Hatching rate

During the experimentation of *Labeo calbasu* the average hatching rate of three breeding trials for both treatments are shown in the Table 3 and 4. The mean rate of fertilization in treatment-1 was recorded 74.5% in three trials and the mean rate of fertilization in treatment-2 was recorded 59.66%. The highest hatching rate (76%) was found in Trial-3 of treatment-1. The lowest hatching rate was 58% recorded in trial-1 for treatment-2. Graphical presentation of hatching rate for both treatments are shown in Fig-3.

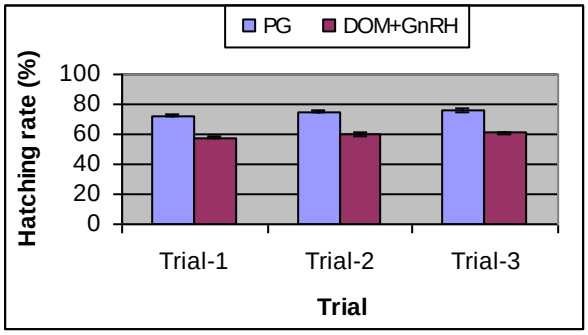

Fig-3. Hatching rate (%) *Labeo calbasu* in different breeding trials

Table-3: Breeding performance of kalibaus (*Labeo calbasu*) with different doses of PG hormone in different breeding trials.

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	100 ± 0.0	75 ± 0.73	72.5 ± 0.56
Trial-2	100 ± 0.0	77 ± 0.73	75 ± 0.73
Trial-3	100 ± 0.0	77.36 ± 0.59	74.50 ± 0.73

Table-4: Breeding performance of kalibaus (*Labeo calbasu*) with different doses of DoM+SGnRH in different breeding trials.

Trials	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)
Trial-1	75 ± 0.73	62.5 ± 0.84	58 ± 0.98
Trial-2	87.5 ± 0.35	64 ± 0.56	60 ± 1.38
Trial-3	83.33 ± 0.35	63.83 ± 0.70	59.66 ± 0.43

First feeding: Although the hatchlings of *Labeo calbasu* retained yolk sack upto 72 h after hatching the larvae started first feeding from 45-55 h of post-hatching at ambient temperature of 26.5-29°C. Boiled egg-yolk was provided as first food for the hatchings.

Discussion

Brood stock management is one of the major aspects in induced breeding of any fish species. In hatchery management, maintenance of brood fishes for the development of modern aquaculture activities has become one of the most important concepts.

Proper care of brood stock is very important for assuring good production of eggs, fry and fingerlings (Robert *et al.*, 1982). The daily and seasonal rates of feeding of brood stock diets have direct effects on fecundity and egg size (Jones and Bromage, 1987, Bromage and Cumaranatunga, 1988). After successful completion of brood stock management with balanced feed comprising adequate amount of protein, lipid, and carbohydrate, especially enriched with vitamin-E the fish *Labeo calbasu* attained gonadal maturity in late April. In the present study, it was found that *Labeo calbasu* bred in April and August. The peak breeding season was May and June and it continued till August. According to Jhingran (1982) the breeding season of the Indian major carps generally start from April and continues to August, with an optimum period between May and June. Ibrahim *et. al.* (1968) reported that temperature ranging from 26.5° to 35.0°C was appropriate for spawning of major carps. Breeding of *Labeo calbasu* was performed at an ambient temperature of 26.5° to 31.1°C.Use of feeds with vitamin mineral premix might have some positive effect for the maturation of fishes. Hoque (1990) reported that diets containing 1% vitamin premix showed better result in all aspects viz., selectively, spawning success, fertilization and hatching rate. Spawning performance of the reared broods indicated that the spawners might have been at their optimal breeding condition. This might be due to good management practices of brood stock which prolomged their breeding season in artificial condition.

In this experiment the induced breeding trials of *Labeo calbasu* were done with pituitary gland (PG) extract and DoM + SGnRH in inducing of *Labeo calbasu*. They achieved good fertilization and hatching rate (73.05% and 60.43%, respectively) by using an initial and a resolving dose of 2.0-3.0 and 5.0-7.0 mg/PG/Kg body weight for female. In this experiment best fertilization and hatching rate (80% and 76% respectively) were gained by using PG in trial-3 for treatment-1. In this study Trial-3 showed best result due to brood fishes which were fully matured. The result in consideration of fertilization and hatching rate was lowest in Trial-1 for the synthetic hormone (DoM+SGnRH). The ovulation occurred in all the injected females within 5.65 to 7.36 hrs after resolving dose to the females at the temperature from 27 to 31°C. But in this experiment ovulation occurred within 6 to 6.30 hrs after resolving dose to females at the temperature from 27 to 31°C.The success of entire operation of induced breeding depends largely on the proper selection of brood fishes. Accomplishment of successful spawning depends on selection of suitable recipient fish at the proper stages of ovarian development and creation of congenial spawning conditions.

The present study was conducted to compare the effects of two inducing agents on the induced breading of *Labeo calbasu*. Three breeding trials were done with PG hormone as treatment-1 and three breeding trials were done with

DOM+GnRH as treatment-2. Brood fishes were collected from the river. The collected broods were reared in the pond, providing special diet up to their maturation. After rearing for certain period the gravid fishes were selected for induced breeding trial. Total 16 broods (8 female and 8 male) were used in each breeding trial for breeding. In case of Treatment-1, the females were injected 1.5mg PG/kg body weight as initial dose and 6 mg PG/kg body weight as resolving dose. On the other hand, males were administered at the rate of 1.5 mg PG/kg body weight. On the contrary in case of Treatment-2, the females and males were injected DOM+GnRH at the rate of 12 mg/kg body weight and 3 mg/kg body weight respectively. In treatment-1 gave the better result in terms of ovulation, fertilizaiton and hatching rate compared to treatment-2. In case of treatment-I, better results were obtained by trial-3 among other trials. From the present study it can be said that, both the inducing agents were effective in respect of overall breeding performance. Although best results were obtained with PG hormone but the entire induced breeding technique could be performed within short time with less labour and cost in case of synthetic agent (DOM+GnRH) than that of PG hormone.

A second experiment was conducted to know the nursery management technique of *Labeo calbasu* in the nursery pond for a period of 60 days. The fry were stocked at the rate of 30g/deci. Wheat bran, mustered oil cake, flour were given as nursery feeds. The mean size of the fry was 2 cm during harvesting.

At present, concerns have been expressed for its conservation through habitat improvement and attempts have been made towards the culture of the fish in the pond. The seed production technology of the species is the prerequisite of development of culture technique. With this end in view, to develop the induced breeding, nursery management of *Labeo calbasu* the present study was undertaken. From the available references along with the present investigation on the induced breeding of the fish comparatively higher percentages of fertilization and hatching were achieved from PG hormone. This can be considered as an effective means for commercial seed production in the hatcheries since cost and quality of hormone is on of the important key factors for a hatchery manger. The results indicated that the hatchery operators can easily breed, rear and supply requisite quantities of *Labeo calbasu* seeds to meet up the demand of the fish farmers. The present study can throw a light for further research on the improvement of induced breeding technique of the fish concerning the hormone application, nursery management techniques of *Labeo calbasu* as well as optimization of the environmental parameters.

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